

**EVALUATION OF COLOUR STABILITY OF FLEXIBLE
DENTURE BASE RESIN IN FOUR COMMONLY
USED FOOD SUBSTANCES**

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In partial fulfilment for the degree of

MASTER OF DENTAL SURGERY



BRANCH I

PROSTHODONTICS AND CROWN AND BRIDGE

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CERTIFICATE

This is to certify that the dissertation titled “**Evaluation of colour stability of flexible denture base resin in four commonly used food substances**” is a bonafide record of work done by **Dr. S. Janani** under my guidance during her postgraduate study period 2009 – 2012.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY**, in partial fulfilment for the degree of **Master of Dental Surgery in Prosthodontics including Crown and Bridge and Implantology**. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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INTRODUCTION

The esthetic appearance of restorations is influenced by colour, translucency, surface glaze, texture and fluorescence. These factors are determined by the illuminant- light source, inherent optical parameters of the restorative materials and the interpretation of the observer. Light waves emitted from the illuminant, interact with the object and are perceived by the observer. This interaction process includes reflection, absorption and transmission of light. The observer receives the light reflected or transmitted by the object and then interprets the results²⁷.

The shade of denture base polymer is selected in clinical situations to match with the natural colour tone of the soft tissues. Colour stability is an important property of a denture base material. It refers to the ability of the denture base material to retain its colour in a specified environment, especially in long term use. It is a required characteristic of denture base polymer, as specified by various national and international standards typically ADA specification no 12.¹ The discoloration of denture base resins results in esthetic problems. The resin should be capable of being tinted or pigmented but there should be no change in colour or appearance of the material subsequent to its fabrication. Most denture base

materials are subject to sorption, a process of absorption and adsorption of liquids dependent upon environmental conditions⁸. Discoloration is possible especially, when the contacting solution is pigmented.

Indian food is known to have a high quantity of ingredients which have a high staining capacity. Hersek N¹⁰ has reported that beverages such as tea, coffee, wine and artificial food colorants like erythrosine, tartrazine and sunset yellow increase the development of stain on clean acrylic surfaces. Absorption and penetration of these colorants into the organic phase of the resin based materials is probably due to the compatibility of the polymer phase with these pigments³⁷. Tea and coffee contain large amount of staining agents like gallic acid and tannins. Denatured proteins and iron from the diet contain thiol groups that provide sulphur and eventually form iron sulphide which is responsible for the stain. Turmeric has been used as a flavouring or colouring agent in a variety of foods. The yellow colour of turmeric is due to curcumin(3%) which is an active substance²². Staining of prostheses by colorants in a service environment may be more largely responsible for colour change than colour instability of the material itself.

Chowdary R³ has reported that the prevalence of edentulism in India varies from 60% to 69% in 25 years and above age group. The incidence of tooth loss increases with age leading to an increase in prevalence of partial edentulism in general population. The removable partial dentures are an economical treatment option in these patients. Due to increased esthetic expectations, non metal clasp dentures using thermoplastic resins have recently become a treatment option for these patients. It is a pressure injected flexible denture base resin that is ideal for partial dentures. The material is a specialized form of nylon in the family of super polyamides that will not deteriorate when it comes into contact with fluids and microorganisms in the oral environment. They possess properties like superior esthetics, reduced potential for allergic reactions, and flexibility due to high elastic nature that decreases the stresses on the abutment teeth¹⁴. They do not require tooth preparation as cast partial dentures and they reduce the chair time required to construct the prosthesis.

The main disadvantage of this material is the lack of rigidity, the essential criteria of removable partial denture and also difficulty in repair but their flexibility allows the design to take full advantage of the undercuts, especially recessed areas of supporting alveolar contours and they provide retention without pressure at the

contact point. It achieves the effect of a stress breaker without attachments, the gingival tissue is gently stimulated under mastication and unnatural stresses on the remaining teeth are substantially reduced^{34,40}. There are only few studies reported on the colour stability of flexible denture base resins. Being a more esthetic alternative to cast partial dentures their colour stability has to be evaluated over clinical use.

AIM AND OBJECTIVE

AIM

The aim of this study was to evaluate the colour stability of flexible denture base resin in four commonly used food substances in India.

OBJECTIVE

The objective of the study was to evaluate the colour stability of flexible denture base resin of two thicknesses (1.0 – 1.5 mm) and (2.0- 2.5 mm) in four commonly used food substances such as coffee, tea, turmeric and sunset yellow dye.

REVIEW OF LITERATURE

Crispin B J et al⁴ in **1979** compared the colour stability of materials used in the fabrication of temporary restorations. Six different temporary restorative materials were tested. Tea, coffee and grape staining solutions were used. It was concluded that rough materials darkened significantly more than polished materials. There was no statistically significant difference in the amount of staining between air cured and pressure cured samples. Based on chemical composition the methyl methacrylate material showed the least darkening followed closely by ethyl methyl methacrylate material.

Khan Z et al¹⁸ in **1987** evaluated the staining potential of the VLC material with a conventional acrylic resin, polymethyl methacrylate. Tea and sodium azide solutions were prepared and staining tests performed. The Triad material showed significantly greater staining than the acrylic resin material in tea solution maintained at 37 and 50 degrees C. The differences in staining characteristics were ascribed to the differences in water sorption exhibited by the two materials, that of Triad VLC being 3.6 times greater than that of conventional acrylic resin denture base material.

May K B et al²³ in **1992** evaluated the colour stability of five denture base acrylic resins (Lucitone Hypro, Acron, Triad, Accelar 20 and Compak 20) and one denture base repair resin (Perm). The samples were subjected to conditions of accelerated ageing to test colour stability. It was found that the colour of Lucitone, Hypro and Acron was least affected by conditions of accelerated ageing. Triad, Accelar 20 and Perm demonstrated noticeable colour changes. Compak 20 had an appreciable colour change and was least colour stable of the materials tested.

Ilmaz B et al¹¹ in **1994** evaluated the colour stability of one light polymerized, three heat polymerized, and three autopolymerized denture base polymers that were exposed to coffee, tea and water at 50 degrees. It was found that coffee and tea stained the denture base materials superficially and brushing with tooth paste and moderate grinding reduced discolouration to an acceptable level. All materials were relatively colour stable when immersed in water at 50 degrees.

May K B et al²⁴ in **1996** evaluated the colour stability of seven conventional and one microwave heat cured denture base material. The samples were subjected to conditions of accelerated ageing in both to test for colour stability. The results revealed that

colour changes occurred after accelerated ageing in both heat cured denture base resins and Argon GC microwave acrylic resins processed with the microwave method.

Wang X et al³⁸ in **1996** evaluated the colour stability of heat activated and chemically activated acrylic resins processed by compression moulding or fluid resin matrix. They concluded that heat activated acrylic resins were more colour stable than chemically activated acrylic resin.

Yannikakis S A et al³⁹ in **1998** evaluated the discolouration effects of coffee and tea on some materials that are commonly used in the fabrication of provisional restorations. It was concluded that provisional restorative materials, staining solutions, and immersion time were significant factors that affected color stability. After immersion for 7 days, all materials showed observable color changes. The composite-based materials, especially light-curing composites, were the least color stable. The coffee solution exhibited more staining capacity than the tea solution.

Hersek N et al¹⁰ in **1999** evaluated the colour stability of five commercially available denture base acrylic resins. The specimens were exposed to 3% erythrosine, tartrazine and sunset yellow solutions. Colour changes were determined with a computer

controlled spectrophotometer after 1, 3 and 6 months of exposure to staining solutions. It was concluded that all materials tested had acceptable colour stability when exposed to the food colorants. The polymethyl methacrylate denture base resins were hydrophilic and attracted more water soluble dyes on the surface and staining occurred as a result of electrostatic charges. The least staining was found to be in the sunset yellow solution as erythrosine and tartrazine have more electrostatically charged groups than the sunset yellow dye.

Lai Y et al²¹ in **2003** compared the colour stability, stain resistance, and water sorption of 4 materials (one silicone, one copolyamide and 2 heat polymerized acrylic resins) commonly used for gingival flange prostheses. The specimens were immersed in coffee and tea solutions. The specimens placed in water and exposed to air served as controls. All flange materials tested demonstrated color stability in air and water. However, the color changes of silicone and copolyamide materials stored in coffee solution for 180 days were greater than 3 NBS (National Bureau of Standards) units, which would be characterized as appreciable and considered clinically unacceptable. The disparity in staining may be partly attributable to different polar properties of the tested materials that affect both the affinity of a material to extrinsic stains and the diffusion of water molecules.

Koksal T et al¹⁹ in **2008** evaluated the colour stability of two brands of porcelain teeth and three brands of acrylic denture teeth. Samples were immersed into three staining drinks as test groups and distilled water as control. Colour change values were determined after 1 day, 1 week, 2 weeks, and 4 weeks of immersion. Instant coffee was found to be the most chromogenic among the solutions tested. Among the materials tested porcelain was found to be more resistant to discoloration. It was concluded that acrylic teeth showed a high degree of colour change and that the amount of colour change for each group increased proportionally with time.

Rodrigues M et al²⁹ in **2009** evaluated the effect of different microwave polymerization cycles on the colour changes of a microwave processed denture base resin after accelerated ageing and immersion in beverages such as coffee, tea, cola and wine. It was concluded that the colour changes of microwave polymerized denture base resin tested were not affected by different polymerization cycles after accelerated ageing or immersion in beverages.

Daniela M et al⁵ in **2010** evaluated the colour stability, surface roughness, surface porosity of acrylic resins for eye sclera polymerized by different heat sources and submitted to accelerated

artificial ageing. Three heat sources short cycle, long cycle, dry heat oven were used. It was concluded that irrespective of the type of heat used for polymerization, there was an intense colour alteration to clinically unacceptable levels when the specimens were subjected to artificial accelerated ageing.

Imirzalioglu P et al¹² in **2010** investigated the effect of four solutions [saliva (control group), saliva+tea, saliva+coffee, saliva+nicotine] on the colour of different denture base acrylic resins (heat-polymerized, injection-molded, autopolymerized) and a soft denture liner. Colorimetric measurements were done on the 1st, 7th and 30th days. Goldstein and Schmitt reported that when ΔE is more than 3.7, it is no longer within the limits of clinical acceptability and it assumes the quality of visual detectability. The color shifts of all test materials were clinically acceptable ($\Delta E < 3.7$) except for soft liner in nicotine, which was not clinically acceptable over time. A decrease in colour difference values was observed for each type of material in tea and coffee solution especially after the 7th day. This was attributed to the removal of accumulated layers from the specimens once they reached a certain thickness. Therefore it was concluded that minimizing drinking of such beverages and use of tobacco, particularly when soft liner is applied, may be advantageous for denture wearers for long-term color stability.

Goiato M C et al⁶ in **2010** evaluated the possible chromatic and microhardness alterations of the flexible resins Ppflex and Valplast in comparison to the conventional acrylic resin Triplex when submitted to accelerated aging. Accelerated aging significantly increased the values of microhardness of the resins tested, with the highest values being observed for the resin Triplex. The resin Valplast presented the greatest chromatic alteration value after accelerated aging, which was significantly different from those of the other resins tested. This was either due to the lower monomer content of Triplex and Ppflex or greater amount of reagents such as benzoyl peroxide in Valplast.

Takabayashi Y et al³⁵ in **2010** examined six thermoplastic resins and conventional acrylic resins and found that clinically noticeable staining may occur on the polyamide resins and polyethylene terephthalate resins. The staining occurred due to the physical penetration of pigments between the molecular lattices or the adsorption of pigments on the surface of the specimens.

Navarro W. F. S et al²⁵ in **2011** determined the colour stability of two heat cured denture base acrylic resins (Lucitone 550, Vipi Cril) and one nylon denture base material (Transflex) by subjecting them to three beverages - coffee, cola and red wine.

Distilled water served as control. It was concluded that the greatest chromatic changes were found in red wine immersion followed by coffee and cola. Transflex displayed staining after immersion in cola. The staining effect of red wine and coffee on the three commercially available resins used as denture base material was found to be at clinically acceptable levels.

MATERIALS AND METHODS

In this study, colour stability of valplast material (Valplast International Corporation, New York, USA) was tested in two thicknesses [20x10 x (1-1.5mm)] and [20 x10 x (2-2.5mm)].

Materials used in the study:

Table 1

Material	Manufacturer
Flexible denture base material – Valplast	Valplast International Corp, New York, USA
Modelling wax	Hindusthan Dental products, Hyderabad, India
Sprue wax	Renfert, Germany
Dental stone	Asian chemicals, Rajkot, Gujarat, India
Processing flask	Valplast International Corp, New York, USA
Ultra Violet visible recording Spectrophotometer, Mac Beth 7000 A	Tokyo, Japan

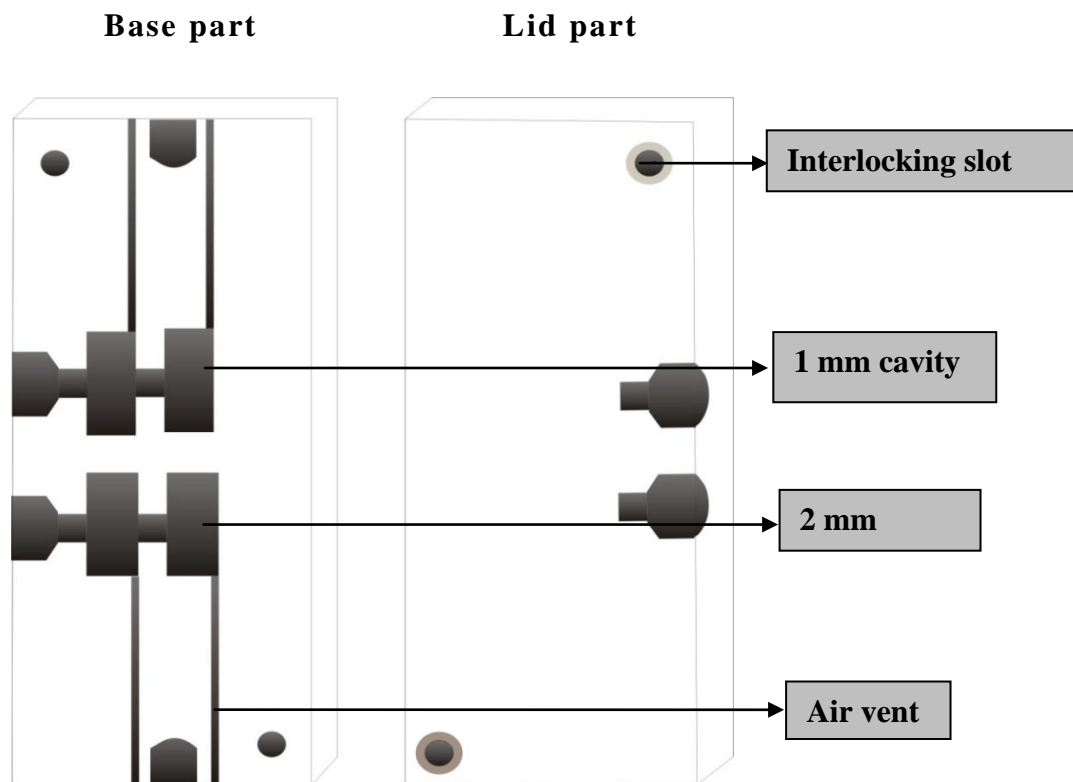
The study methodology followed was:

1. Metal die for wax pattern fabrication
2. Preparation of the wax pattern
3. Processing and finishing of the samples
4. Grouping of samples
5. Preparation of staining solutions
6. Staining procedure
7. Spectrophotometric analysis of colour stability

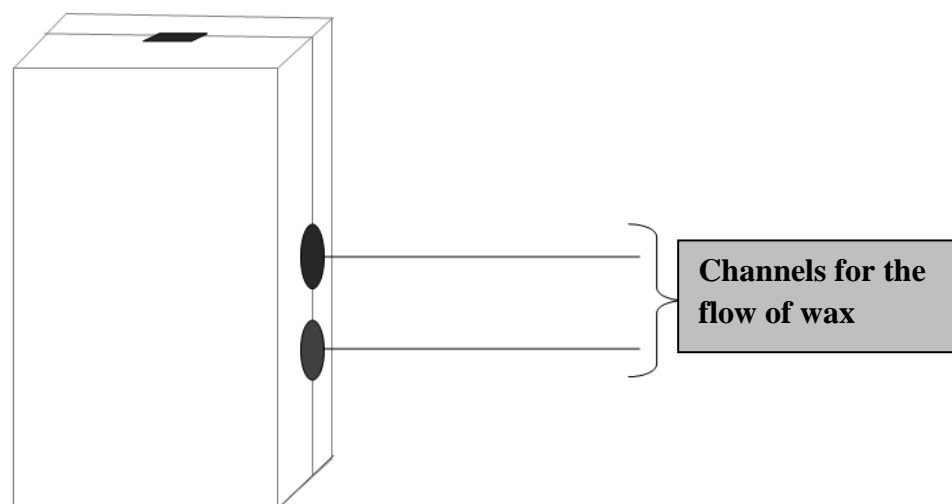
Metal die for wax pattern

An aluminum metal mould was made for making wax patterns. The mould was made in two parts – base and lid. The base part contained 4 rectangular cavities, two cavities with internal diameter of 20x10x (1-1.5 mm) and two cavities with internal diameter of 20x10x (2-2.5 mm). Air vents were made along the side of the cavities to prevent the formation of air bubbles during the preparation of wax patterns. The lid was provided with two interlocking slots to seat accurately on the base. On locking, the base and the lid formed a channel through which molten wax (Modelling wax, Hindustan Dental Products, Hyderabad, India) was poured to make the wax patterns.

A Schematic representation of the mould



Assembled metal die



Preparation of wax pattern

The base and the lid portions of metal mould was coated with petroleum jelly and locked together. Modelling wax (Hindustan Dental Products, Hyderabad, India) was melted and poured through the two channels of the mould. The metal mould was immersed in water and the wax was allowed to cool. Once cooled the two parts of the mould were separated and the patterns were carefully removed.

Processing and finishing of the samples

The samples were processed by injection moulding technique by using a flask specially designed by the manufacturer. Wax sprues were attached and the wax patterns were invested in the lower part of the dental flask using dental stone (Type 3 gypsum).

After the stone had set, separating medium was applied and space maintainer for the cartridge was placed. The counter part of the flask was placed on the base part and the dental stone was poured. After the stone was set, the flask was placed in boiling water for 4-6 minutes. The space maintainer and the patterns were gently removed from the investment material and dewaxed thoroughly. Separating medium was applied and flask allowed to cool to the room temperature.

The valplast cartridge was placed in the furnace and preheated to a temperature of 287.70 °C (550° F) for 11 minutes. The stone moulds were exposed under heat lamps and was uniformly heated for 15 to 20 minutes to a temperature of 80°C, to avoid any premature freezing of the molten nylon as it entered the mould cavity under pressure. The metal injector was placed in position and along with the cartridge containing melted Valplast, they were placed on to the injection unit. The molten Valplast was then forced into flask using a plunger, the injection moulding pressure being maintained at 5 bars for 3 min and then the assembly was removed and disengaged. The flask was bench-cooled for 20 min and then deflasked. The blanks were removed from the moulds and the sprues were removed with a Valplast specific cutting disc. The surfaces of the specimens were polished using Valplast specific polishing compounds according to the manufacturer's instructions.

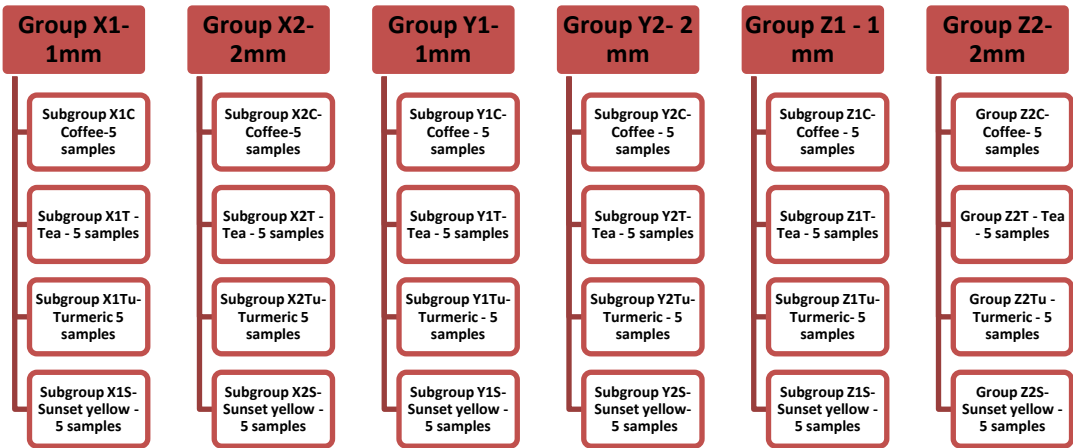
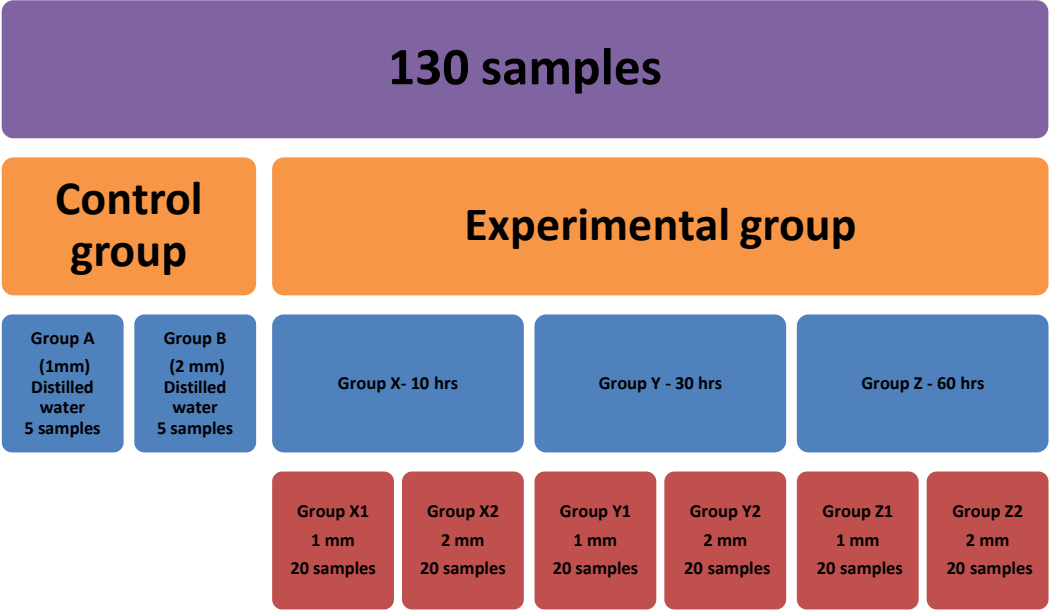
Grouping of the samples

A total of 130 samples were made. It was divided into control group (10 samples) and experimental group (120 samples). The control group was further divided into Group A (1mm – 5 samples) and Group B (2mm-5 samples). The experimental group was divided into Group X (10 hrs duration), Group Y (30 hrs duration), Group Z (60 hrs duration).

Group X was further divided into Group X1 (1 mm), Group X2 (2mm). Group X1 was further divided into Subgroup X1C (Coffee-5 samples), Subgroup X1T (Tea-5 samples), Subgroup X1Tu (Turmeric- 5 samples), Subgroup X1S (Sunset yellow-5 samples). Group X2 was further divided into Subgroup X2C (Coffee-5 samples), Subgroup X2T (Tea-5 samples), Subgroup X2Tu (Turmeric-5 samples), Subgroup X2S (Sunset yellow-5 samples).

Group Y was further divided into Group Y1 (1 mm), Group Y2 (2mm). Group Y1 was further divided into Subgroup Y1C (Coffee - 5 samples), Subgroup Y1T (Tea - 5 samples), Subgroup Y1Tu (Turmeric- 5 samples), Subgroup Y1S (Sunset yellow - 5 samples). Group Y2 was further divided into Subgroup Y2C (Coffee - 5 samples), Subgroup Y2T (Tea - 5 samples), Subgroup Y2Tu (Turmeric- 5 samples), Subgroup Y2S (Sunset yellow - 5 samples).

Group Z was further divided into Group Z1 (1 mm), Group Z2 (2mm). Group Z1 was further divided into Subgroup Z1C (Coffee - 5 samples), Subgroup Z1T (Tea - 5 samples), Subgroup Z1Tu (Turmeric- 5 samples), Subgroup Z1S (Sunset yellow - 5 samples). Group Z2 was further divided into Subgroup Z2C (Coffee-5 samples), Subgroup Z2T (Tea-5 samples), Subgroup Z2Tu (Turmeric- 5 samples), Subgroup Z2S (Sunset yellow - 5 samples).



Preparation of the staining solutions

The colour stability was assessed in four commonly used food ingredients.

Table 2

Food substances	Manufacturer
Coffee, Bru	Hindustan Unilever Ltd, Mumbai, Maharashtra, India
Tea, Three Roses	Brooke Bond Pvt Ltd, Kolkata, West Bengal, India
Turmeric, Sakthi	Sakthi Pvt Ltd, Erode, Tamil Nadu, India
Sunset yellow	Venus Chemicals and flavours, Chennai, Tamil Nadu, India
Distilled water	Prasad Associate, Coimbatore, Tamil Nadu, India.

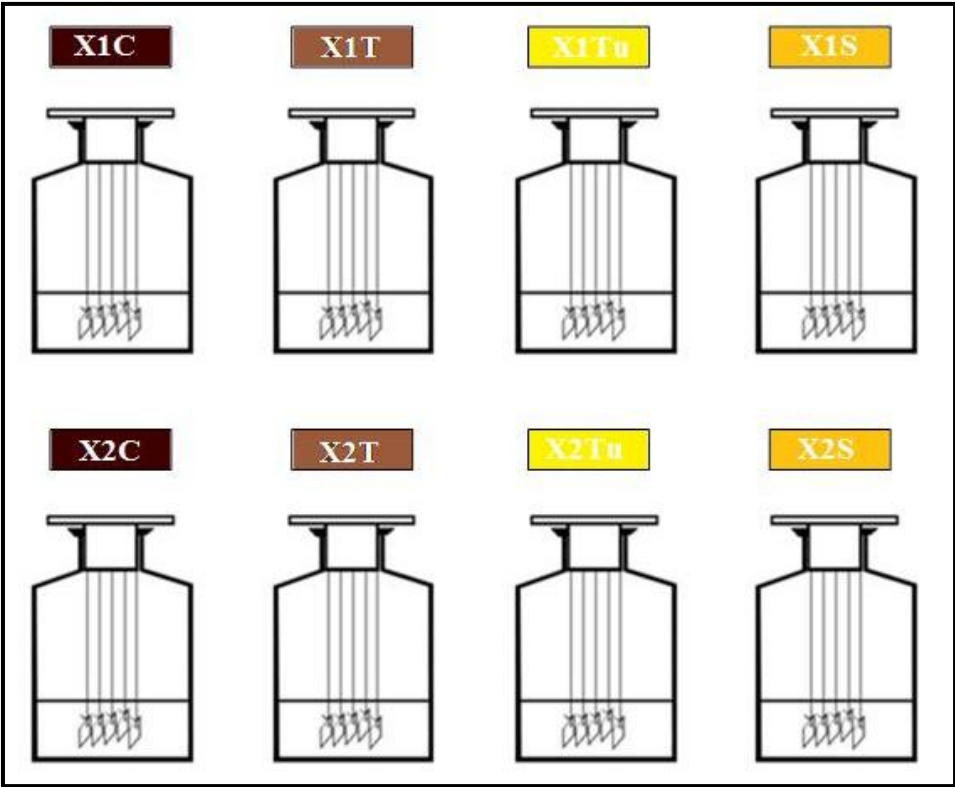
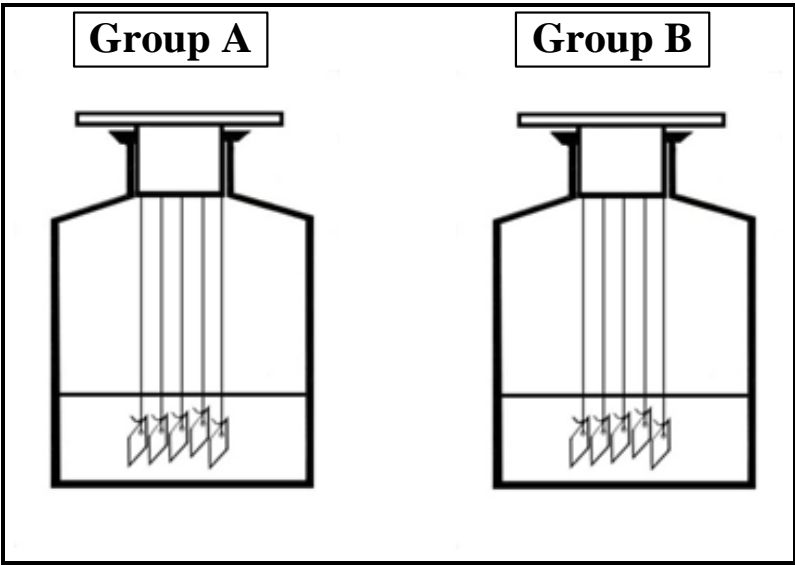
Coffee solution was prepared by dissolving 2 g of coffee (Bru) in 200 ml of distilled boiling water for 2 minutes. Tea solution was prepared by dissolving 2 g of Tea (Three Roses) in 200 ml of distilled boiling water for 2 minutes. Turmeric solution was prepared by dissolving 1 g of turmeric (Sakthi) in 200 ml of distilled boiling water for 2 minutes. Sunset yellow solution was prepared by dissolving 0.05 g of sunset yellow dye in 200 ml of distilled boiling water for 2 minutes. The solutions were filtered to remove the dust. Fresh solutions were prepared once in a week.

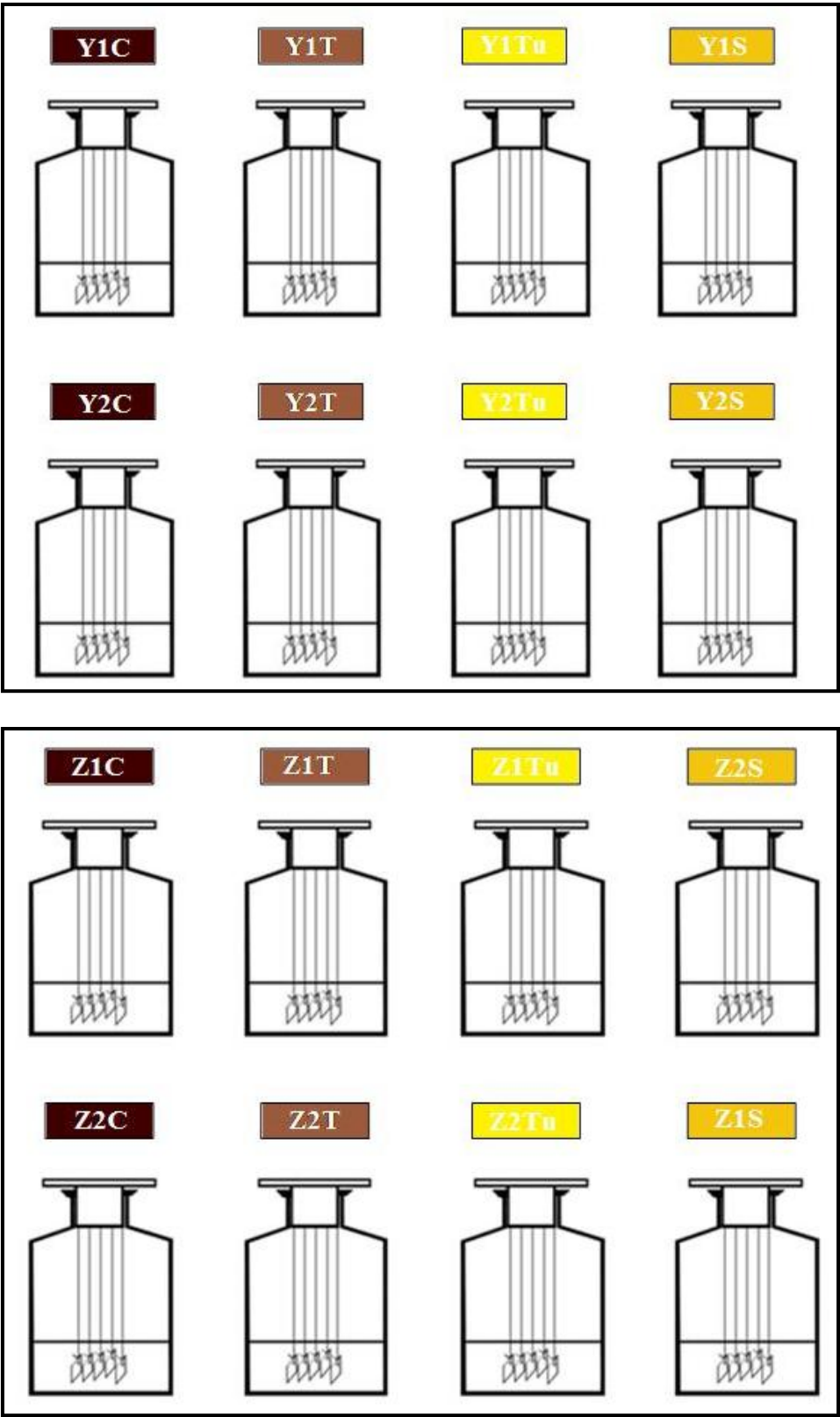
Staining procedure

The control group samples (Group A and Group B) were stored in distilled water in separate glass jars. The staining solutions prepared were allowed to cool to room temperature and stored in glass jars in dark. The solutions for the three groups - Group X, Y and Z, were stored in separate containers. The glass jars were labelled indicating the solution, duration and the thickness of the samples. Holes were made in all the 130 samples and they were suspended by means of threads in the solutions. They were immersed in the test solution for a period of one hour /day after which the specimens were removed, rinsed in distilled water and stored in distilled water in dark at room temperature to simulate the oral conditions. This procedure was done for 10 days, 30 days and 60 days for Groups X, Y and Z respectively.

Schematic representation of the staining procedure

- A-Control – 1 mm
- B-Control – 2 mm
- X- 10 hrs Y- 30 hrs Z- 60 hrs
- X1-1mm X2-2mm Y1-1mm Y2-2 mm Z1-1mm Z2-2 mm
- C-Coffee T – Tea Tu – Turmeric S- Sunset yellow



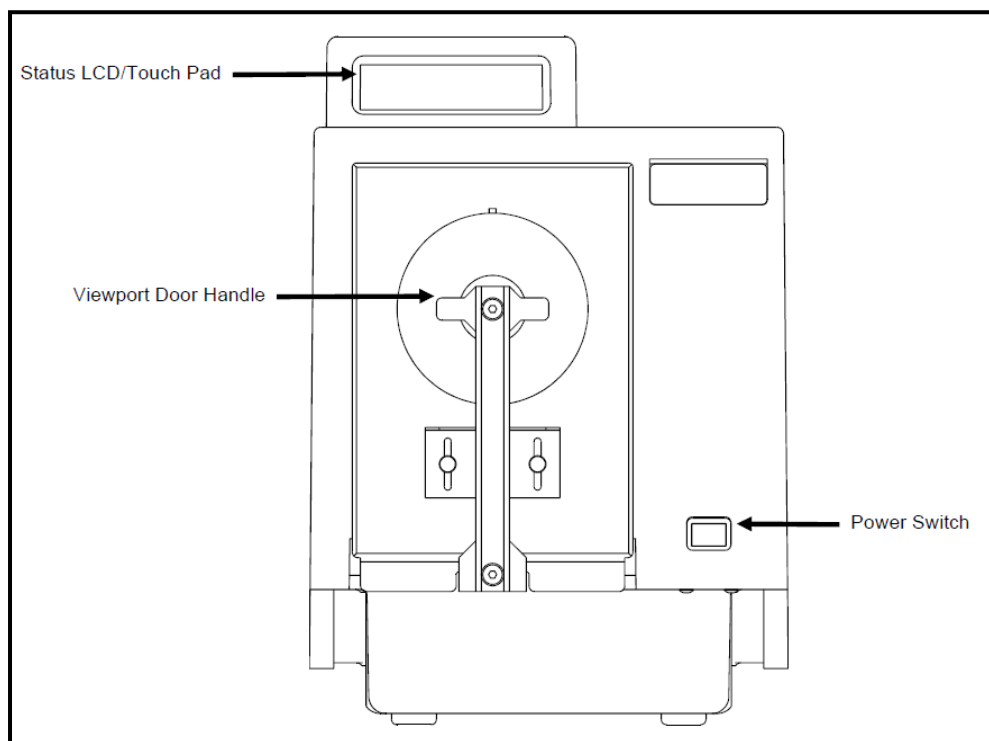


Spectrophotometric colour analysis of colour stability

On the day of evaluation, the samples were removed from distilled water, and packed in plastic bags, labelled and taken for colour analysis. The colour analysis of the samples was done with ultraviolet visible recording spectrophotometer (Macbeth 7000A) using the CIE colour lab system.

Basic principle of spectrophotometer

To perceive colour accurately, three elements are required: light source, sample and observer. The spectrophotometer contains artificial daylight D65 that is used as the standard light source for colour evaluation. The operator measures a standard colour through the lens of the spectrophotometer. The instrument then reports the results to the computer, which, in turn, performs a mathematical calculation. The sample colour to be evaluated is then analysed by the same process and compared to the standard. The instrument used to view the sample is the observer, i.e the lens of the spectrophotometer.

Schematic representation of the spectrophotometer – front view**Colour analysis of Valplast samples**

Each sample was dried thoroughly by blotting with tissue paper before colour analysis. It was placed in the view port of the spectrophotometer and L, a and b values of each sample were measured. Three readings were made for each sample and the average output per sample was given automatically by the spectrophotometer using the CIE colour lab system.³¹ A total of 130 readings were taken, 10 readings for the control group and 120 readings for the experimental group.

The values of the control group were taken as L1 (standard), a1 (standard) and b1 (standard) for 1 mm samples and L2 (standard), a2 (standard) and b2 (standard) for 2 mm samples. Similarly values of the test sample was taken as L1 (sample), a1 (sample) and b1 (sample) for the 1 mm samples and L2 (sample), a2 (sample) and b2 (sample) for the 2 mm samples.

The colour difference ΔE for 1mm sample was calculated using the formula:

$$\Delta E (1\text{mm}) = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

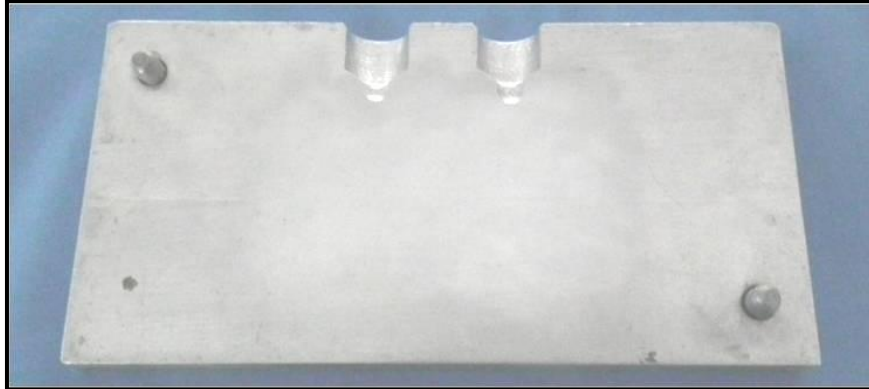
where $\Delta L = L1 \text{ (sample)} - L1 \text{ (standard)}$, $\Delta a = a1 \text{ (sample)} - a1 \text{ (standard)}$, $\Delta b = b1 \text{ (sample)} - b1 \text{ (standard)}$

The colour difference ΔE for 2mm samples was calculated using the formula:

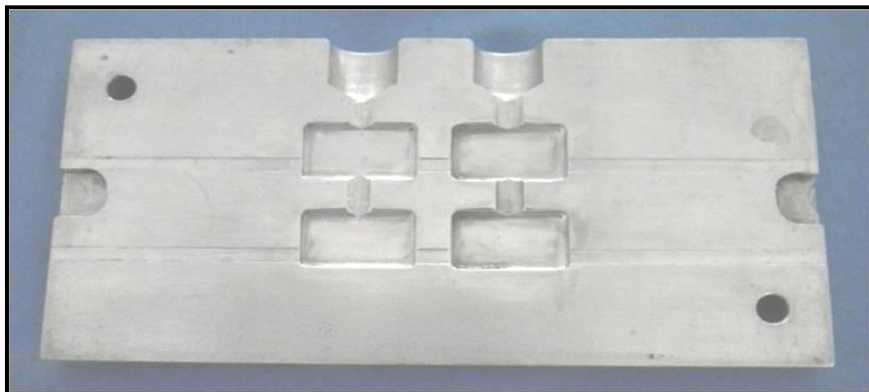
$$\Delta E (2\text{mm}) = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

and $\Delta L = L2 \text{ (sample)} - L2 \text{ (standard)}$, $\Delta a = a2 \text{ (sample)} - a2 \text{ (standard)}$, $\Delta b = b2 \text{ (sample)} - b2 \text{ (standard)}$.

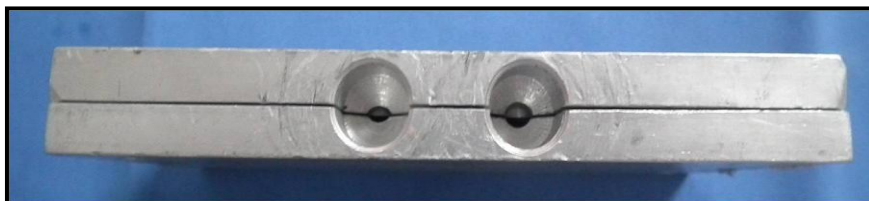
METAL DIE



Lid part

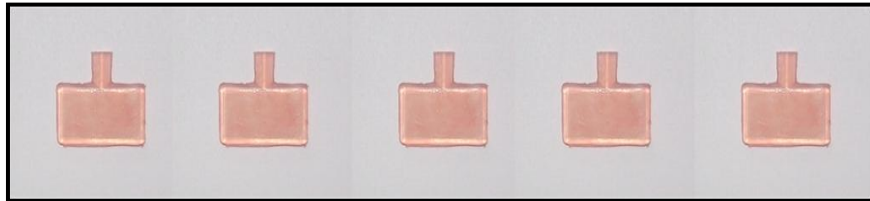


Base part



Base and lid interlocked

Group A (Control- 1mm)



Group B (Control – 2 mm)

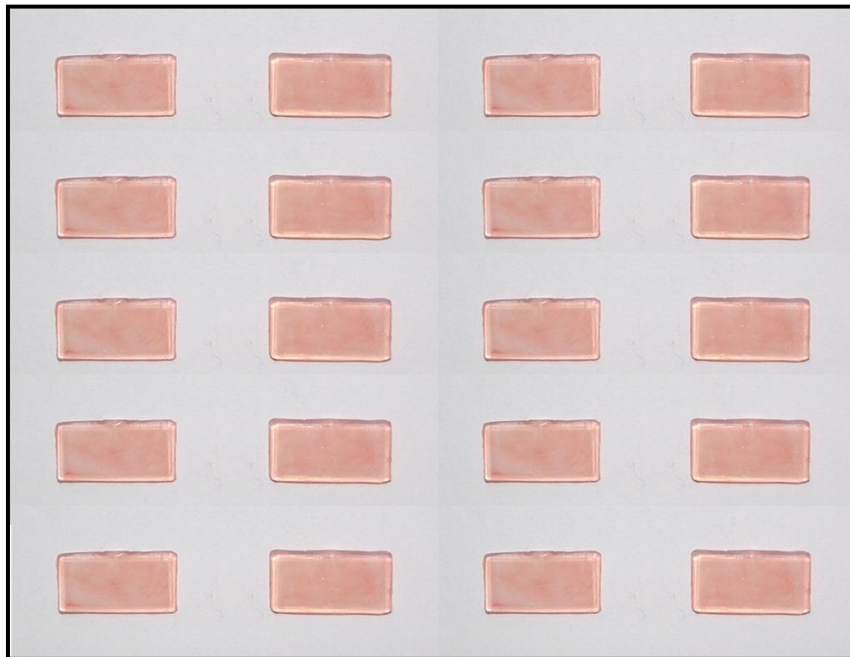


STAINING PROCEDURE

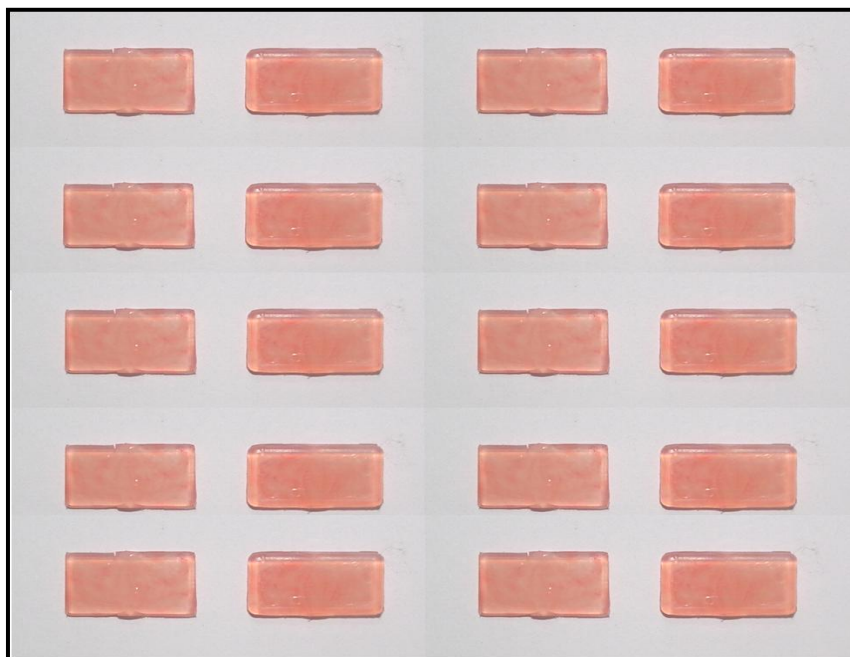
Group A and Group B (Immersion in distilled water)



Group X1(10 hrs – 1mm)



Group X2(10 hrs – 2 mm)



Group X1

(1 mm samples- Immersion in staining solutions for 10 hours)

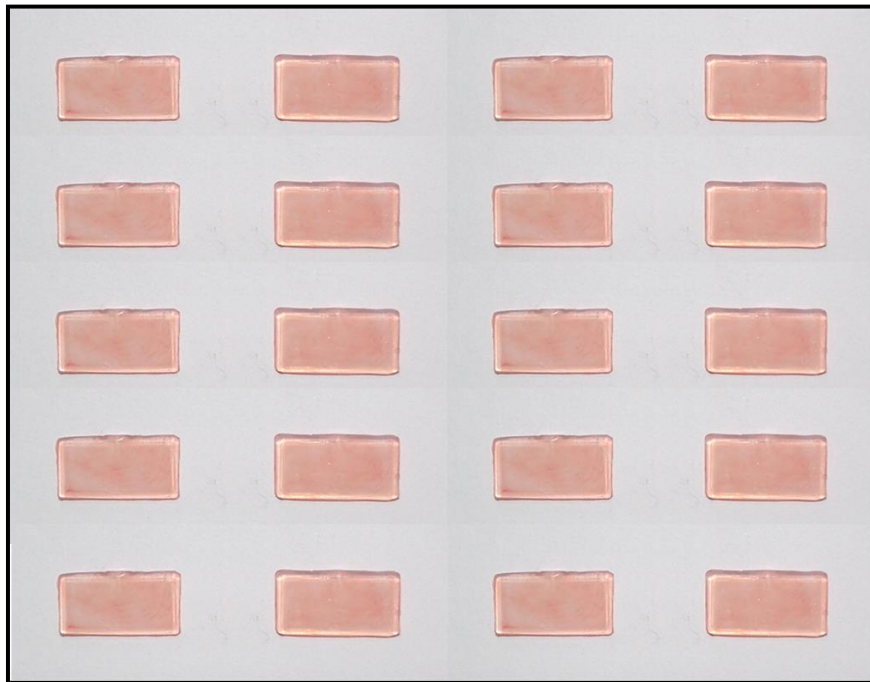


Group X2

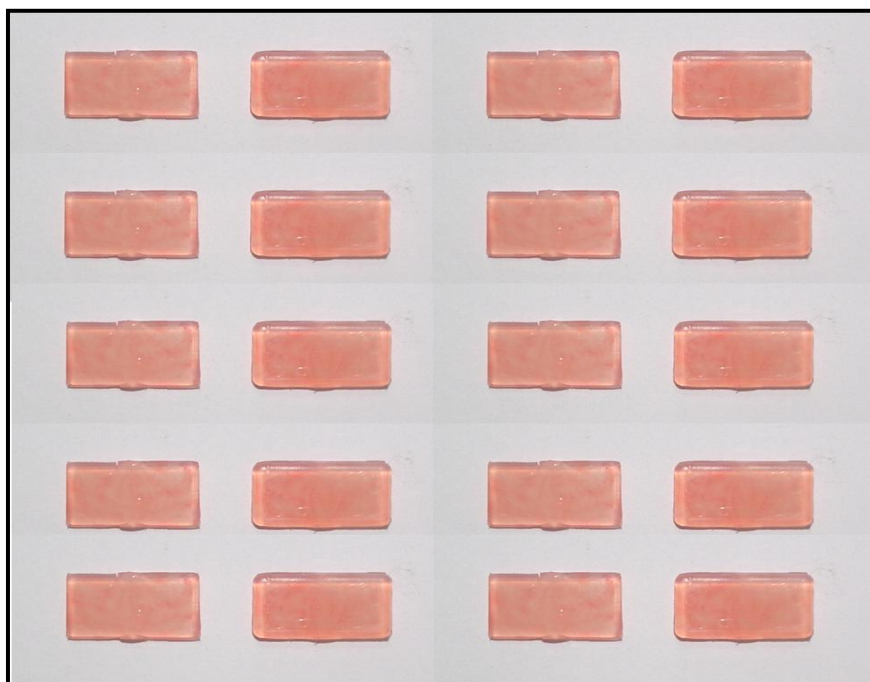
(2 mm samples- Immersion in staining solutions for 10 hours)



Group Y1 (30 hours – 1mm)



Group Y2 (30 hours – 2mm)



Group Y1

(1 mm samples- Immersion in staining solutions for 30 hours)

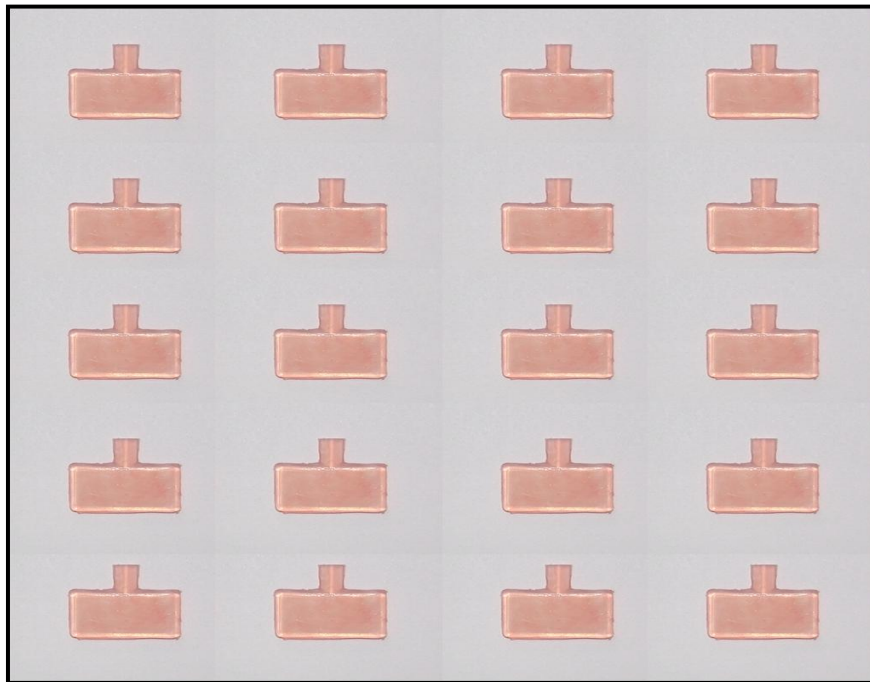


Group Y2

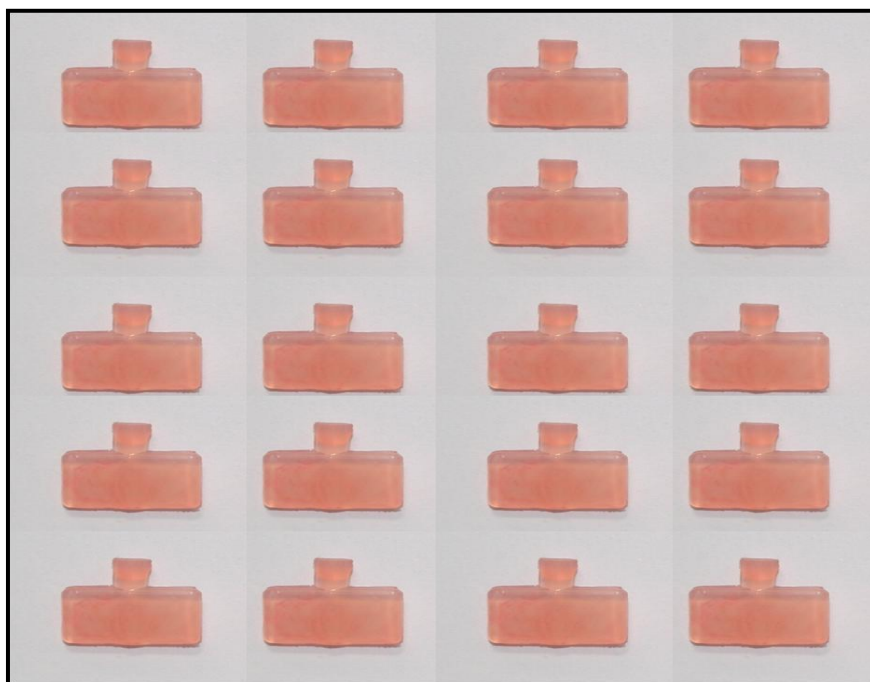
(2 mm samples- Immersion in staining solutions for 30 hours)



Group Z1 (60 hours – 1mm)



Group Z2 (60 hours – 2 mm)



Group Z1

(1 mm samples- Immersion in staining solutions for 60 hours)



Group Z2

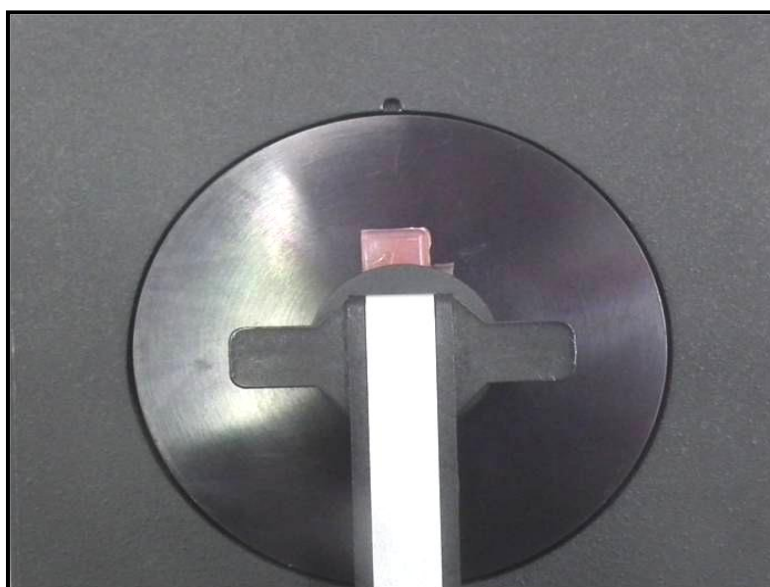
(2 mm samples- Immersion in staining solutions for 60 hours)



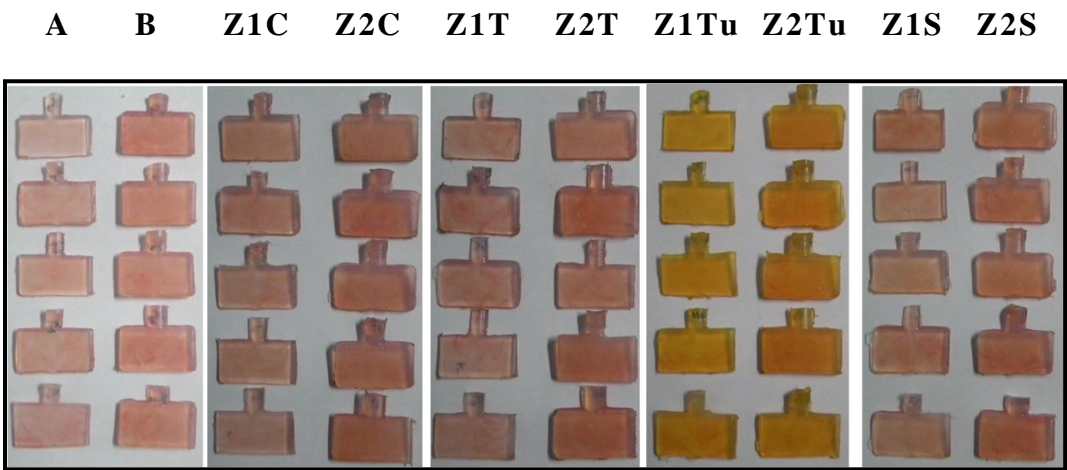
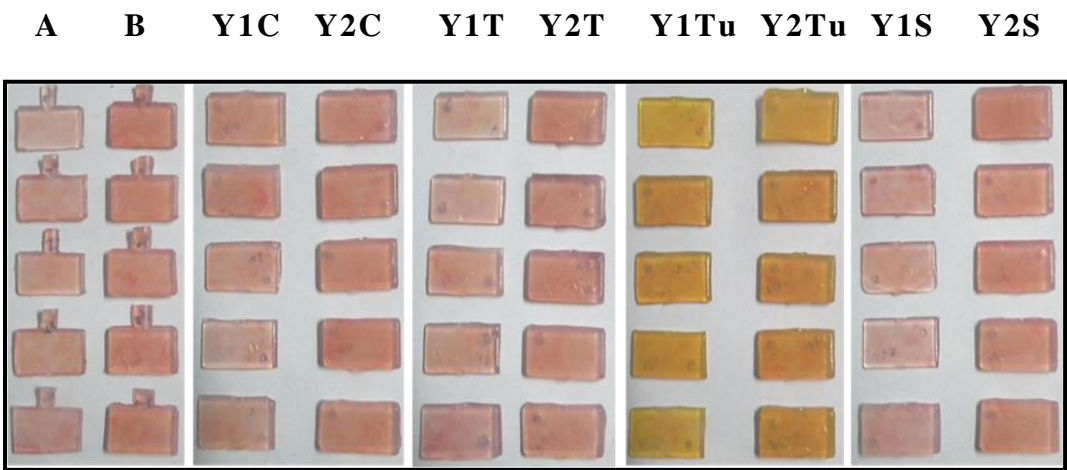
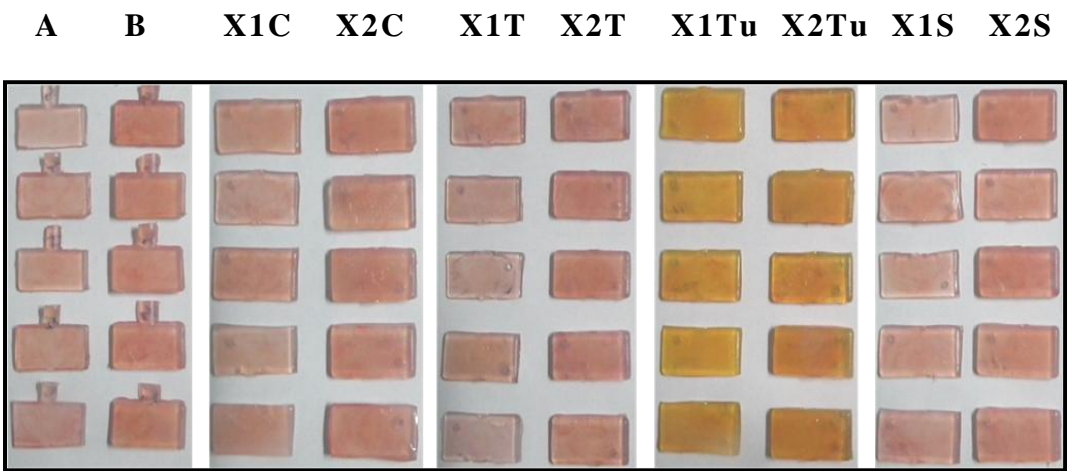
Macbeth 7000 A spectrophotometer



Spectrophotometric colour analysis of the samples



Colour change observed in the experimental groups
(Groups X, Y and Z)



RESULTS

The colour difference ΔE values of 120 samples (Group X, Group Y, Group Z) are presented.

Table 3

10 hrs group	Coffee		Tea		Turmeric		Sunset yellow	
	1mm	2 mm	1mm	2 mm	1mm	2 mm	1 mm	2 mm
1	1.30	3.14	0.93	2.65	21.19	20.57	1.13	3.18
2	1.79	2.19	2.33	2.04	22.42	20.41	1.29	2.88
3	1.59	4.39	0.65	2.13	21.47	16.88	1.36	2.39
4	1.55	2.12	1.54	2.83	20.26	18.95	1.36	2.36
5	1.5	3.11	1.96	2.14	21.33	18.98	1.35	4.25
30 hrs group								
1	2.68	3.65	1.35	2.63	19.40	18.76	1.02	3.92
2	2.21	3.61	1.74	3.05	19.08	22.75	1.61	2.20
3	1.70	3.34	2.20	3.50	20.0	23.87	1.70	2.21
4	2.19	2.70	1.96	3.51	19.40	22.40	2.34	2.97
5	2.19	3.03	2.44	2.68	19.42	22.37	1.01	2.39
60 hrs group								
1	1.62	3.13	0.97	2.62	18.16	18.81	2.20	2.23
2	1.16	4.18	1.33	3.04	17.89	18.19	1.98	2.87
3	1.21	4.17	1.37	2.94	17.14	18.83	1.52	2.89
4	0.65	2.08	1.50	2.65	17.31	18.52	1.65	3.16
5	1.11	1.46	1.45	2.63	16.27	18.55	1.83	3.18

Statistical Analysis

The mean and standard deviations of the colour change ΔE of valplast material of two thicknesses in coffee, tea, turmeric and sunset yellow solutions are as follows

Table 4

Thickness		1mm			2 mm		
Duration	Solution	Mean	Std. Deviation	Std.Error of Mean	Mean	Std. deviation	Std.Error of Mean
10 hrs	Coffee	1.55	0.18	0.08	2.99	0.92	0.41
	Tea	1.48	0.70	0.31	2.36	0.36	0.16
	Turmeric	21.33	0.77	0.34	19.16	1.49	0.66
	Sunset Yellow	1.30	0.10	0.04	3.01	0.77	0.35
	Total	6.42	8.85	1.98	6.88	7.33	1.64
30 hrs	Coffee	2.19	0.35	0.16	3.27	0.40	0.18
	Tea	1.94	0.42	0.19	3.07	0.43	0.19
	Turmeric	19.46	0.33	0.15	22.03	1.93	0.86
	Sunset yellow	1.54	0.55	0.25	2.74	0.73	0.33
	Total	6.28	7.82	1.75	7.78	8.50	1.90
60 hrs	Coffee	1.15	0.35	0.15	3.0	1.22	0.55
	Tea	1.32	0.21	0.09	2.78	0.20	0.09
	Turmeric	17.35	0.73	0.33	18.58	0.26	0.12
	Sunset yellow	1.84	0.27	0.12	2.87	0.38	0.17
	Total	5.42	7.09	1.58	6.81	7.00	1.57

The mean and standard deviation was calculated and subjected to One-Sample Kolmogorov-Smirnov Test. This test was done to find out whether the given distribution is normal or not. To test this, a null hypothesis was formed that the observed data followed normal probability distributions.

Table 5

One-Sample Kolmogorov-Smirnov Test					
		Solution			
		Coffee	Tea	Turmeric	Sunset yellow
		Delta E	Delta E	Delta E	Delta E
N		30	30	30	30
Normal Parameters (a, b)	Mean	2.3582	2.1587	19.6527	2.2143
	Std. Deviation	1.01784	.75496	1.90189	.84199
Most Extreme Differences	Absolute	.158	.129	.149	.117
	Positive	.158	.094	.149	.117
	Negative	-.079	-.129	-.090	-.082
Kolmogorov-Smirnov Z		.865	.709	.814	.643
Asymp. Sig. (2-tailed)		.443	.696	.521	.803
a Test distribution is Normal.					
b Calculated from data.					

From table 5 it was inferred that all asymptotic significance values were greater than 0.05 (5% level of significance), so the null hypothesis was accepted for all sets of data. The results obtained were normally distributed for all groups of solution and analysis of variance technique could be used for this data set.

Univariate analysis of variance

Analysis of variance (ANOVA) is used to uncover the main and interaction effects of categorical independent variables (called "factors") on an interval dependent variable. In this study ANOVA test was used to determine the effect of duration of the study, solution and thickness of the material on the colour difference ΔE values.

To determine the relation between the variables, the following null hypotheses were generated.

Null hypothesis H_{01} : There is no significant effect of DURATION, on the mean values of colour difference.

Null hypothesis H_{02} : There is no significant effect of the type of SOLUTION on the mean values of colour difference.

Null hypothesis H_{03} : There is no significant effect of difference in THICKNESS on the mean values of colour difference.

Null hypothesis H_{04} : There is no significant interaction between DURATION and SOLUTION on the mean values of colour difference.

Null hypothesis H_{05} : There is no significant interaction between DURATION and THICKNESS on the mean values of colour difference.

Null hypothesis H_{06} : There is no significant interaction between SOLUTION and THICKNESS on the mean values of colour difference.

Null hypothesis H_{07} : There is no significant interaction between TIME, THICKNESS AND SOLUTION on the mean values of colour difference.

Table 6-Univariate analysis of variance

Tests of Between-Subjects Effects					
Dependent Variable: Delta E					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	6940.843(a)	23	301.776	568.843	.000
Intercept	5220.800	1	5220.800	9841.135	.000
DURATION	17.026	2	8.513	16.047	.000
SOLUTION	6819.741	3	2273.247	4285.039	.000
THICKNESS	37.403	1	37.403	70.504	.000
DURATION * SOLUTION	31.274	6	5.212	9.825	.000
DURATION * THICKNESS	6.433	2	3.217	6.063	.003
SOLUTION * THICKNESS	3.666	3	1.222	2.304	.082
DURATION * SOLUTION * THICKNESS	25.299	6	4.217	7.948	.000
Error	50.929	96	.531		
Total	12212.572	120			
Corrected Total	6991.772	119			
a R Squared = .993 (Adjusted R Squared = .991)					

From table 6 it was inferred that:

For the variable DURATION Sig. values were less than 0.01, so the null hypothesis H_{01} was rejected. Therefore the effect of DURATION was statistically significant in colour difference at 1% level of significance.

For the variable SOLUTION Sig. values were less than 0.01, the null hypothesis H_{02} was rejected. Therefore the effect of SOLUTION is statistically significant in colour difference at 1% level of significance.

For the variable THICKNESS Sig. values were less than 0.01, the null hypothesis H_{03} was rejected. Therefore the effect of THICKNESS was statistically significant in colour difference at 1% level of significance.

For the variables DURATION and SOLUTION Sig. values were less than 0.01, the null hypothesis H_{04} was rejected. Therefore the interaction effect of DURATION and SOLUTION was statistically significant in colour difference at 1% level of significance.

For the variables DURATION and THICKNESS, Sig. values were less than 0.01, the null hypothesis H_{05} was rejected. Therefore

the interaction effect of DURATION and THICKNESS was statistically significant in colour difference at 1% level of significance.

For the variables SOLUTION and THICKNESS, Sig. values were greater than 0.01, the null hypothesis H_{06} was accepted. Therefore the interaction effect of SOLUTION and THICKNESS was not statistically significant in colour difference at 1% level of significance.

For variables DURATION, SOLUTION and THICKNESS, Sig. values were less than 0.01, the null hypothesis H_{07} was rejected. Therefore the interaction of the effect of DURATION, SOLUTION and THICKNESS was statistically significant in colour difference at 1% level of significance.

Post Hoc Study

From the Univariate Analysis of variance test it was determined that the effect of DURATION, SOLUTION and THICKNESS and their interactions were significant.

Post Hoc test was used in conjunction with ANOVA to determine which specific group was statistically different from other group.

Duration of the study

In Post Hoc test for the duration of the study, the following pairs were compared.

10 hrs and 30 hrs, 10 hrs and 60 hrs, 30 hrs and 60 hrs.

Table 7

Multiple Comparisons						
Dependent Variable: Delta E						
Scheffe						
PAIRS		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) Duration of Study	(J) Duration of Study				Lower Bound	Upper Bound
10 hrs	30 hrs	-.3822	.16287	.069	-.7872	.0227
	60 hrs	.5361(*)	.16287	.006	.1312	.9411
30 hrs	60 hrs	.9184(*)	.16287	.000	.5134	1.3233

The mean difference is significant at the .05 level.

Homogenous subsets

Table 8

Delta E Scheffe			
	N	Subset	
Duration of Study		1	2
60 hrs	40	6.1111	
10 hrs	40		6.6473
30 hrs	40		7.0295
Sig.		1.000	.069

From table 7 and 8, it was inferred that colour difference at 60 hours duration was lower than that of the other durations.

Type of Solution

In Post hoc test for the four solutions (Coffee, Tea, Turmeric and Sunset Yellow) used in the study, the effect of each solution was compared with the other three solutions.

Table 9

Multiple Comparisons						
Dependent Variable: Delta E						
Scheffe						
PAIRS		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) Solution	(J) Solution				Lower Bound	Upper Bound
Coffee	Tea	.1995	.18806	.771	-.3357	.7347
	Turmeric	- 17.2945(*)	.18806	.000	- 17.8297	- 16.7593
	Sunset yellow	.1438	.18806	.900	-.3913	.6790
Tea	Turmeric	- 17.4940(*)	.18806	.000	- 18.0292	- 16.9588
	Sunset yellow	-.0557	.18806	.993	-.5908	.4795
Turmeric	Sunset yellow	17.4383(*)	.18806	.000	16.9032	17.9735

The mean difference is significant at the .05 level.

Homogenous subsets

Table 10

Delta E Scheffe			
	N	Subset	
Solution		1	2
Tea	30	2.1587	
Sunset yellow	30	2.2143	
Coffee	30	2.3582	
Turmeric	30	-	19.6527
Sig.		.771	1.000

From table 9 and 10 it is inferred that turmeric solution produces greater colour change than the other solutions.

The NBS parameter is important to relate the amount of colour change (ΔE) recorded by the spectrophotometer to a clinical environment. The data were converted to National Bureau of Standards units (NBS units) through the equation, $\text{NBS units} = \Delta E \times 0.92$, where critical remarks of colour differences could be expressed in terms of NBS units.¹⁰

The colour change values of experimental valplast samples exposed to food colorants for 10 hrs, 30 hrs and 60 hrs duration according to National Bureau of Standards Unit system

Table 11

	Coffee		Tea		Turmeric		Sunset yellow	
	1mm	2mm	1mm	2mm	1 mm	2 mm	1mm	2mm
Group X (10 hrs)	1.43	2.75	1.36	2.17	19.62	17.63	1.19	2.77
Group Y (30 hrs)	2.01	3.0	1.78	2.82	17.90	20.27	1.42	2.52
Group Z (60 hrs)	1.06	2.76	1.21	2.56	15.96	17.09	1.69	2.64

Trace – 0.0 – 0.5



Slight – 0.5- 1.5



Noticeable – 1.5- 3.0



Exceeding noticeable range - > 3.0



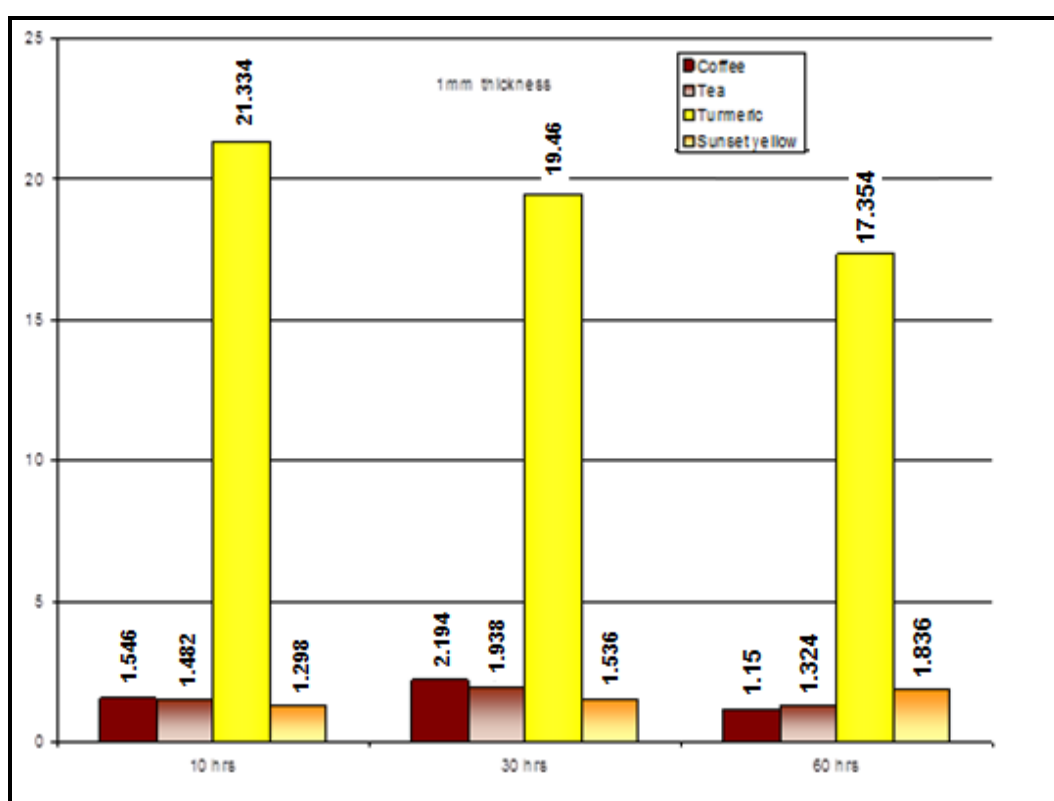
From Table 11 it was inferred that subgroups coffee, tea and sunset yellow of 1mm thickness in Group X, subgroup sunset yellow of 1mm thickness in Group Y, subgroups coffee and tea of 1mm thickness in Group Z produced slight colour change values. (0.5- 1.5)

The subgroups coffee, tea and sunset yellow of 2 mm in Group X, subgroups coffee and tea of 1mm, coffee, tea and sunset yellow of 2 mm in group Y, subgroups sunset yellow of 1mm, coffee, tea and sunset yellow of 2 mm in Group Z produced noticeable colour change values.(1.5-3.0)

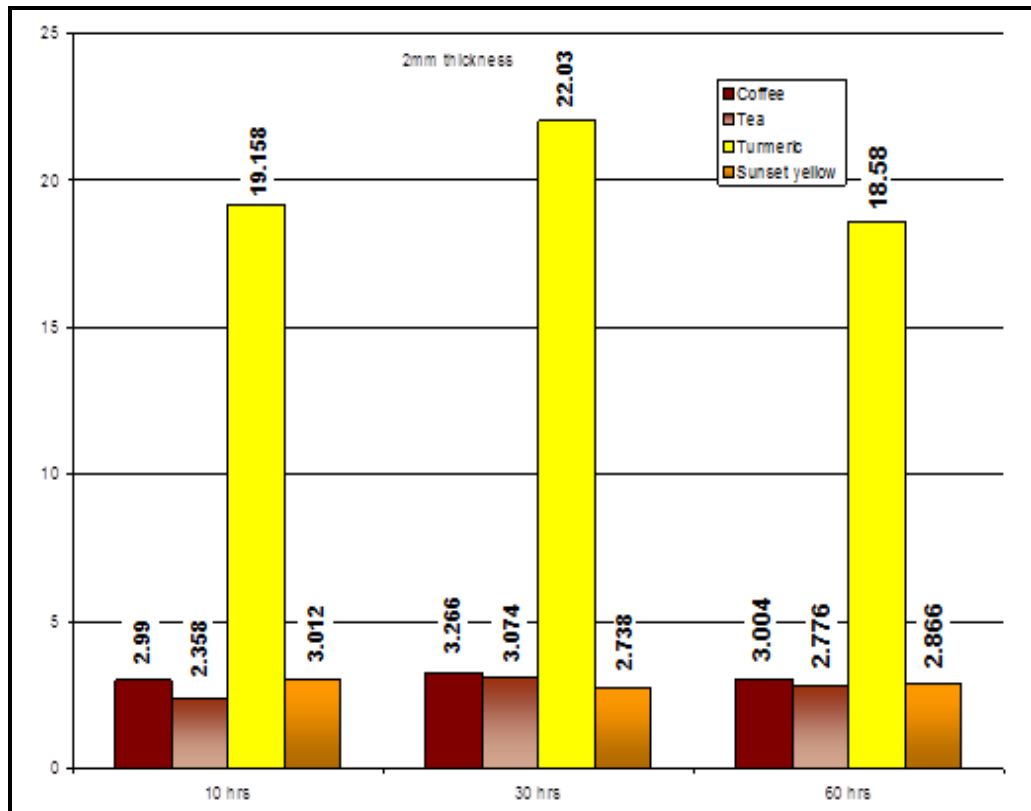
The subgroup turmeric of group X, group Y and group Z exceeded the noticeable range. (>3.0)

Graphs

Colour difference (ΔE) of experimental valplast samples (1 mm) exposed to food colorants for 10 hrs, 30 hrs and 60 hrs duration.



Colour difference (ΔE) of experimental valplast samples (2mm) exposed to food colorants for 10 hrs, 30 hrs and 60 hrs duration



DISCUSSION

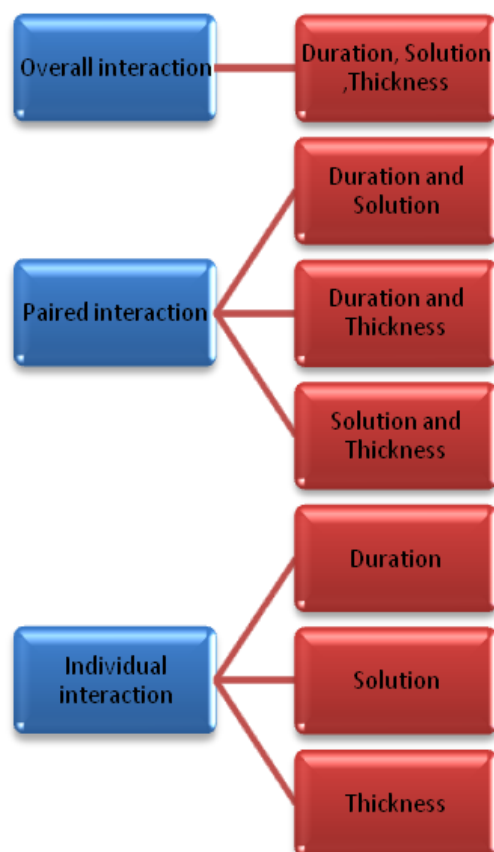
The aim of this study was to evaluate the colour stability of the flexible denture base material valplast of two thicknesses (1-1.5mm) and (2-2.5mm) in commonly used food substances in India. The application of nylon-like materials for the fabrication of dental appliances is seen as an advance in dental materials. These flexible denture base materials are stable and exhibit high creep resistance, high fatigue endurance, excellent wear characteristics and solvent resistance¹⁶. Thermoplastic resins typically have very little or almost no free monomer in the material. A significant percentage of the population is allergic to free monomer and these materials offer an alternative treatment for these individuals^{28,9}. In addition, thermoplastic materials have almost no porosity, which reduces biologic material build up, odours, stains and exhibit higher dimensional stability. All of these factors become important when producing long-term provisional prostheses during implant or complex restorative cases, or when used for permanent removable appliances².

Goiato M C et al⁶ evaluated the possible chromatic and microhardness alterations of the flexible resins and concluded that Valplast presented the greatest chromatic alteration value after

accelerated aging, which was significantly different from those of the other resins tested.

Typically, the thermoplastic resins are more flexible and stronger than their traditional counterparts, but their colour stability has to be evaluated over time.

The interactions of the factors influencing the colour stability in this study were analyzed in the following manner.



Overall interaction of factors influencing colour stability

The results from this study showed that the effect of the overall interaction of the duration of immersion of the samples, the solution used and thickness of the sample were statistically significant in determining the colour stability of valplast material ($p < 0.01$).

Paired interaction of factors influencing colour stability

When the factors were compared in pairs to find out further, their interaction in determining the colour stability of valplast material, the following findings were noticed.

- The interaction effect of DURATION AND SOLUTION was statistically significant ($p < 0.01$) in producing colour change.
- The interaction effect of DURATION AND THICKNESS was also statistically significant ($p < 0.01$) in producing colour change.
- But the interaction effect of SOLUTION AND THICKNESS was not statistically significant ($p > 0.01$).

Individual interaction of factors influencing colour stability***Duration***

The effect of DURATION was statistically significant in producing colour change ($p < 0.01$). Group X, Y and Z showed the following feature:

There was no statistically significant difference in colour stability between Group X and Group Y ($p>0.05$), but there was statistically significant difference between Group X and Group Z ($p<0.05$) and Group Y and Group Z ($p<0.05$). The average ΔE values of Group Z were lower than Group X and Group Y which was in accordance with the previous studies as related to coffee and tea.

Keskin¹⁷ evaluated the colour stability of polymethyl methacrylate denture base polymers after immersion in coffee and tea solutions for 7 days and reported that there was an initial increase and then a decrease in the discolouration values of the materials.

Imirzalioglu P et al¹² studied the effect of tea, coffee and nicotine on the colour of different denture base acrylic resins after 1, 7 and 30 days. A decrease in colour difference values was observed for each type of material in tea and coffee solution especially after the 7th day. This was attributed to the removal of accumulated layers from the specimens once they reached a certain thickness and similar finding was seen in this study.

Solution

The effect of the type of SOLUTION was statistically significant in producing colour change ($p < 0.01$).

The results have also shown that there was no statistically significant difference between subgroups coffee, tea and sunset yellow solution ($p > 0.05$), where as the difference between turmeric solution and each of the other solution was statistically significant ($p < 0.05$). So turmeric solution produced greater colour change than the other solutions.

Thickness

The effect of THICKNESS was statistically significant in producing colour change ($p < 0.01$).

This was an important finding in this study that suggested that the thickness of the material influenced the colour stability of valplast material. The average ΔE of the 1mm samples was 6.037667 and the average of the 2 mm samples was 7.15425.

The colour change could be caused by intrinsic and extrinsic factors. The intrinsic factors involve discoloration of the materials itself with alteration in its matrix. It occurs with aging as a result of physical-chemical conditions such as thermal and humidity changes.

Extrinsic factors such as absorption and adsorption of stains may also cause discolouration.³⁶

The denture base materials absorb liquid slowly over a period of time due to the polar properties of the resin molecules. However it has been proved that the mechanism is diffusion of water molecules that penetrate according to the laws of diffusion. Other factors responsible for colour instability are, infiltration, surface roughness, chemical degradation by use, oxidation during double carbon reactions, producing peroxide compounds and continuous formation of pigments due to degradation of products²⁰.

Goiato M C et al⁶ reported that Valplast presented a greater amount of reagents such as benzoyl peroxide. Some studies that compared the chromatic alterations of auto-polymerizing and heat-polymerized acrylic resins observed greater chromatic instability for the auto-polymerizing resins since these present a great amount of additional reagents such as benzoyl peroxide. This reagent remains after polymerization and may alter the material's color.

Takayabashi Y et al³⁵ reported that valplast materials exhibit less contact angle and are hydrophilic. Polyamides tended to have inherently high water sorption that occurred among the molecular chains due to the high hydrophilicity of the numerous amide bonds

forming the main chains of the polyamide resin. It is also thought that the higher the amide group concentration, the greater the water sorption. Other factors could be the concentration of the staining solution used and the quality of the stain. All the materials tested contained chromophores which are known to be easily polarized. The PA-type materials also contain auxochromes which, in combination with chromophores and free radicals in solution, may result in staining.

A common characteristic of beverages like wine, tea, and coffee is the presence of tanning agents, which have strong chromogenic potential²⁶. Tea flavins in tea leaves are reported to be the cause of discolouration. Caffeine and caffeic acid in coffee causes discolouration. The staining potential of turmeric is due to the known high colorant nature and natural staining capacity of turmeric. The yellow colour of turmeric is due to the active substance curcumin (4-5 %)²². The sunset yellow dye is a water soluble azo dye which has charged and ionisable groups in their chemical structures.¹⁰ The thermoplastic resins are hydrophilic that attracted more water soluble dyes on the surface and staining occurred as a result of electrostatic charges.

In this study the dimension of the samples were determined to meet the requirements of the measuring instrument, ultraviolet visible recording spectrophotometer as done in a previous study¹⁰ that tested polymethylethacrylate resin in three food colorants.

The concentration of the solution was based on an average intake of coffee, tea, turmeric and sunset yellow per day. The average intake of coffee and tea were 500 mg twice or thrice per day. The concentration used was 1% for coffee and tea which was in accordance with the previous studies¹². The maximal intake of sunset yellow in various food substances is 1 mg/ kg body weight.¹³ The normal intake level of turmeric is 400- 600 mg twice or thrice per day.

The samples were immersed in the solutions for a period of 1 hour per day, for 10 days, 30 days and 60 days for the groups X, Y and Z that simulated the clinical use of 1 month, 3 months and 6 months by the patient.

Color changes were characterized using the Commission Internationale d'Eclairage L*a*b* color space (CIE L*a*b*)³³. Basically, the CIELAB system compares a sample to a standard and makes a numerical determination based on the perceived color difference. The "lightness" of a sample is represented by the symbol

"L*" and this value is based on the percent of light reflectance. The "a." value refers to the red shade/green shade color difference. The yellow shade/blue shade value is designated by the letter "b*." The colour difference is calculated by the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ where $\Delta L = L(\text{sample}) - L(\text{standard})$, $\Delta a = a(\text{sample}) - a(\text{standard})$, $\Delta b = b(\text{sample}) - b(\text{standard})$.³¹

Johnson and Kao¹⁵ stated that if ΔE is less than 1, the chromatic alteration is slight, and between 1 and 2 is considered as clinically acceptable. **Goldstein and Schmitt**⁷ reported that when ΔE is more than 3.7, the colour change could be visually detected and becomes clinically unacceptable. **Ruyter et al**³⁰ has reported ΔE values higher than 3.3 to be clinically unacceptable¹².

In this study turmeric solution produced the greatest colour change values with ΔE greater than 3.3 in 10 hours, 30 hours and 60 hours duration in both 1 mm and 2mm thicknesses and exhibited visual detectability and was therefore considered to be clinically unacceptable. All the other solutions except turmeric produced ΔE values less than 3.3 and were considered to be in the clinically acceptable range.

SUMMARY AND CONCLUSION

The objective of this study was to evaluate the colour stability of flexible denture base resin (Valplast) in two different resin thicknesses (1-1.5 mm and 2.0- 2.5 mm) in four commonly used food substance such as coffee, tea, turmeric and sunset yellow dye.

Within the limitations of the study, the following conclusions were drawn,

1. The thickness of the sample, duration of immersion of the samples, the solution used, and their interactions were significant in producing colour change.
2. Though there was an initial increase in colour difference values, there was a gradual decrease after 30 hour duration.
 ΔE values of the 60 hours group were lower than 10 hours and 30 hours group suggesting a gradual increase in colour stability from 30 hours to 60 hours duration.
3. The turmeric solution produced greater colour change than the other solutions ($\Delta E > 3.3$)

4. According to the National Bureau of Standards units, samples of 1 mm thickness immersed in coffee, tea and sunset yellow for 10 hours, sunset yellow for 30 hours and in coffee and tea for 60 hours showed slight colour change values.(0.5- 1.5)

The samples of 1 mm thickness immersed in coffee and tea for 30 hours, sunset yellow for 60 hours and the samples of 2 mm thickness immersed in coffee, tea and sunset yellow for 10 hours, coffee, tea and sunset yellow for 30 hours, coffee, tea and sunset yellow for 60 hours showed noticeable colour change values. (1.5 – 3.0)

The samples of both 1mm and 2mm thicknesses immersed in turmeric solution for 10 hours, 30 hours and 60 hours exceeded the noticeable range.(>3.0) and considered to be clinically unacceptable.

The present study is an invitro study where the interaction of saliva with the food colorants was not examined. Further research is required to evaluate the colour stability in invivo situation and in longer durations of clinical use.

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ANNEXURE

**The L, a, b values of Control group and Experimental group
(130 samples)**

Group	L value	a value	b value
Group A			
1	35.29	7.62	0.53
2	34.29	5.74	0.10
3	35.61	6.52	0.15
4	35.37	6.69	0.17
5	35.02	6.48	0.09
Average	L1-35.11	a1-6.61	b1- 0.2
Group B			
1	32.27	9.29	-0.62
2	32.27	9.29	-0.63
3	32.31	9.50	-0.45
4	32.97	8.93	-0.84
5	32.56	9.95	0.06
Average	L2-32.46	a2-9.39	b2-0.52

Group X1C			
1	34.87	8.32	-0.49
2	36.53	8.30	-0.74
3	35.93	8.53	-0.60
4	35.70	7.80	-0.98
5	36.62	7.26	0.05
Group X2C			
1	35.14	8.04	-0.33
2	34.34	8.57	-0.69
3	36.62	9.82	-0.80
4	34.30	8.85	-0.20
5	35.37	9.30	-0.95
Group X1T			
1	35.18	8.21	-0.09
2	36.70	5.97	-0.06
3	35.53	7.89	-0.06
4	36.34	6.64	0.13
5	36.66	6.51	-0.18

Group X2T			
1	34.62	8.24	-1.27
2	34.13	8.55	-0.18
3	34.36	9.09	-0.98
4	34.62	8.17	0.51
5	34.27	9.29	0.15
Group X1Tu			
1	38.43	-0.87	19.28
2	38.65	-2.53	20.19
3	39.17	0.44	19.93
4	37.66	-1.39	18.48
5	41.43	-0.21	23.13
Group X2Tu			
1	39.47	-0.45	18.23
2	37.92	0.59	17.14
3	35.84	2.12	14.19
4	36.29	2.94	9.49
5	37.23	0.07	15.17

Group X1S			
1	35.41	8.58	-0.06
2	36.05	8.05	-0.43
3	34.92	7.00	-0.63
4	36.49	8.11	0.13
5	35.30	7.54	-0.83
Group X2S			
1	34.98	10.34	0.66
2	34.89	8.44	0.05
3	33.60	9.59	-0.31
4	34.49	10.01	-0.07
5	36.48	9.81	-0.95
Group Y1C			
1	36.50	5.43	-0.27
2	36.66	7.07	-1.07
3	35.96	8.18	-1.00
4	36.00	7.93	-0.32
5	36.33	7.13	-1.34

Group Y2C			
1	35.63	10.67	-0.18
2	34.24	8.89	-0.64
3	35.38	9.78	0.49
4	34.27	10.60	0.64
5	35.00	8.32	-1.53
Group Y1T			
1	34.43	8.64	0.33
2	35.86	6.38	-0.56
3	33.74	9.09	0.02
4	36.46	5.68	-0.37
5	35.48	7.76	-0.18
Group Y2T			
1	34.70	8.48	-0.04
2	35.29	8.85	-0.59
3	35.58	8.14	-0.67
4	33.48	9.39	-0.03
5	34.91	8.87	-0.45

Group Y1Tu			
1	37.65	-0.70	17.90
2	35.76	0.38	18.18
3	37.47	-1.20	18.34
4	35.55	0.79	18.68
5	35.72	0.48	18.13
Group Y2Tu			
1	36.16	1.93	16.19
2	38.01	-0.84	18.92
3	38.57	-2.54	19.13
4	37.40	-0.41	18.91
5	34.79	3.19	14.98
Group Y1S			
1	36.11	7.13	0.12
2	36.17	6.49	-0.23
3	36.23	8.08	-0.81
4	34.45	6.02	-0.96
5	36.01	7.02	0.15

Group Y2S			
1	35.88	8.19	-1.71
2	34.41	9.36	-1,16
3	34.23	10.01	0.12
4	34.94	9.70	-1.87
5	34.56	8.85	-0.10
Group Z1C			
1	35.99	6.93	1.01
2	35.35	6.79	0.57
3	35.31	6.98	0.32
4	35.01	6.48	0.25
5	36.08	6.22	0.63
Group Z2C			
1	29.51	4.51	-0.71
2	33.81	8.39	-0.97
3	36.23	8.19	0.15
4	34.32	9.07	-0.91
5	34.22	8.93	-0.52

Group Z1T			
1	35.89	7.26	-0.15
2	35.67	6.43	0.06
3	35.61	6.36	0.95
4	35.63	6.48	-0.39
5	35.85	6.28	0.59
Group Z2T			
1	34.73	8.51	-0.17
2	35.20	8.48	-0.62
3	34.87	7.99	-0.13
4	35.03	8.41	-0.59
5	34.78	8.22	-0.66
Group Z1Tu			
1	37.72	-1.72	15.92
2	37.29	-0.13	15.63
3	38.14	-1.39	14.89
4	37.01	-0.26	14.66
5	37.28	-0.68	16.83

Group Z2 Tu			
1	32.29	-1.90	14.50
2	36.87	0.60	14.02
3	32.28	-1.89	14.50
4	36.19	-0.43	14.64
5	37.58	-0.18	16.83
Group Z1S			
1	36.17	6.50	2.17
2	35.49	6.13	1.62
3	36.75	6.38	2.30
4	35.74	7.32	2.09
5	36.68	6.60	1.16
Group Z2S			
1	33.38	9.39	1.02
2	34.40	7.99	0.79
3	34.40	7.94	0.81
4	32.25	6.31	0.48
5	26.86	3.13	-0.34